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SEROLOGIC SURVEY OF SELECTED ZOONOSES AND CANINE VIRAL PATHOGENS IN GRIZZLY BEARS (*URSUS ARCTOS*) AND BLACK BEARS (*URSUS AMERICANUS*) FROM ALASKA

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Six cent quarante quatre échantillons de sérum récoltés sur 480 ours grizzly et 40 ours bruns d'Alaska entre 1988 et 1991 furent testés pour la présence d'anticorps contre diverses zoonoses et infections virales canines. La prévalence chez les ours grizzly était de 0% pour la parvovirose, 8.3% (40/480) pour la maladie de Carré, 14% pour l'hépatite infectieuse, 16.5% pour la brucellose, 19% pour la tularémie et 47% pour la trichinose. La prévalence en anticorps variait chez les ours bruns de 0% pour la parvovirose et la maladie de Carré à 27.5% pour la trichinose et 32% pour la tularémie. La prévalence en anticorps anti-brucelliques et anti-tularémiques était identique (2.5%) pour les grizzly et les ours bruns de la même région. Des différences notables de prévalence furent observées selon l'origine et l'age des ours. Les anticorps anti-Carré et anti-hépatite infectieuse étaient surtout présents chez les ours de l'Ile Kodiak et de la péninsule d'Alaska. Les anticorps anti-brucelliques étaient principalement observés chez les ours de l'ouest et du nord de l'Alaska, et les anticorps anti-tularémiques chez les ours des régions centrales et arctiques. Un gradient croissant Sud-Nord en anticoprs anti-trichinosiques fut observé. La séroprévalence augmentait avec l'age pour la plupart des infections testées, mais pour quelques-unes aucun anticorps n'était détectable chez les grizzly <2,5 ans. Les grizzly apparaissent être d'excellentes sentinelles pour la surveillance épidémiologique des zoonoses en Alaska.

INTRODUCTION

There are an estimated 30,000 grizzly bears (*Ursus arctos*) and 140,000 black bears (*Ursus americanus*) in Alaska (USA). As predators and scavengers, bears may come in contact with agents of zoonotic diseases. Serologic evidence of infection by *Brucella* spp. in Alaskan grizzly bears has been reported previously and in black bears from other parts of North America. Bears can be infected by *Francisella tularensis*, the agent of tularemia, which is endemic in Alaska. However, no extensive serosurvey of this infection in Alaskan bears has been performed. Trichinosis is also known to be endemic in Alaska, and antibody prevalence >50% have been reported in brown bears (*Ursus arctos*). Antibodies to canine distemper virus (CDV) and canine parvovirus (CPV) have been reported from giant pandas (*Ailuropoda melanoleuca*) in China, and more recently for distemper from polar bears (*Ursus maritimus*) from Alaska and Russia. Evidence of canine infectious hepatitis (CIHV) antibodies in Alaskan bears has also been reported, but no information is available on seroprevalence in Alaskan grizzly and black bears of canine distemper virus and canine parvovirus, infections which have been reported in Alaskan and Canadian wolves. The objective of this study was to determine the seroprevalence of various zoonotic agents and canine viruses in Alaskan bears.

MATERIAL AND METHODS

Personnel of the Alaska Department of Fish and Game and the U.S. Fish and Wildlife Service captured 480 grizzly bears (GB) and 40 black bears (BB) in the course of performing population ecology studies between 1988 and 1991. Sampling was opportunistic and some bears were captured more than once; 644 serum samples were available for testing. 76 blood samples were collected from 40 BB in Interior Alaska on the Tanana Flats, south of Fairbanks. The 568 GB blood samples were collected from 8 different areas : In southern Alaska, 79 samples were collected on Kodiak Island, and 86 samples from the Alaska Peninsula (Katmai Coast (38 samples) and Black Lake (48 samples)). In Interior Alaska, 53 samples were collected in the Tanana Flats, Denali Park, and Fairbanks areas. In Western Alaska, 40 samples came from Seward Peninsula and 99 from Noatak river drainage. In Northern Alaska,133 samples came from northwestern Alaska, 6 from north central Alaska, and 72 from northeastern Alaska. Blood samples were collected by femoral, saphenous or cephalic venipuncture. Serum was separated by centrifugation and stored at -20°C until tested. Samples were collected from 25 (63%) of the 40 BB more than once : 4 BB had blood taken 4 times; 3 BB, 3 times, and 18 BB twice. Among the GB, samples were obtained 3 times from 11 GB and twice from 67 GB. Serologic tests were performed at the Veterinary Public Health Lab., Davis, California (brucellosis, tularemia and trichinosis) and at the Virology Lab., Rhône-Mérieux, Lyon, France (CDV, CPV, CIHV). Sera were tested for evidence of antibodies to : (1) *Francisella tularensis* (tularemia) using a commercial slide agglutination test. Any titer [3] 1:20 was considered positive, and positive serums were retested in order to eliminate non-specific reaction. (2) *Brucella* spp. by buffered positive serum acidified card antigen test, and, and positive serumsamples were also retested. (3) *Trichinella spiralis*, by enzyme linked immunosorbent assay (ELISA). The ELISA was based on the official USDA method for pseudorabies, modified as follows. Briefly, 50µl per well of a *Trichinella spiralis* ES antigen (5µg/ml) produced by the Vet. Diagnostics Lab. , Ames, Iowa, USA at 1:1000 dilution in carbonate bicarbonate buffer, pH 9.6, was bound to 96-well flat bottom microtitration plates (Linbro/Titertek plates (Flow Laboratories, Inc., McLean, Va, USA) by overnight incubation at 4°C. Bear sera were diluted 1:40 in tris buffer (pH 7.4) containing 5% skim milk and 0.05% tween 20 and 0.01% bovine serum albumine fraction V (Sigma Chemical Co., St. Louis, Mo, USA). The peroxidase conjugate was a raccoon anti-bear antibody at 1:500 (raccoon anti-bear IgG, Antibodies Inc., Davis, Ca, USA). The substrate

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was 2,2'-azino-bis(3-ethylbenzthiazoline-6-sulfonic acid;ABTS) (Sigma Chemical Co.). The reaction was stopped after 30 min with 100 µl of a 0.1 M solution of hydrofluoric acid (pH 3.3). Each plate contained known positive and negative control sera. The positive control serum was selected from a bear which was both positive by bentonite flocculation and ELISA (optical density (O.D.)=1.0), and the negative controls were selected from 3 bears both negative by ELISA (O.D.<0.1) and bentonite flocculation. Each serum was tested in duplicate and the mean of the two absorbance values calculated. Microtitration plates were read at 410 and 450 nm respectively for test and reference on a microelisa autoreader (MR 5000, Dynatech Laboratories, Inc. Chantilly, Va, USA). The cut-off point for a positive test was determined at O.D.= 0.3, which was the mean O.D. of the negative control population (all bears from Kodiak Island) plus 3 standard deviations (SD) .(4) canine parvovirus by hemagglutination inhibition. A viral suspension of 4 hemagglutining units in a 0.05 ml buffer was added to the various dilutions of the serum samples to be tested for 1 hr at room temperature. Then, 0.05 ml of a suspension of $3x10^7$ SRBC were added in the plate wells and left overnight at 4^0C. The reaction was read the next day. (5) canine distemper virus by competitive ELISA. Briefly, 100µl per well of capture monoclonal antibodies at a 1:1,500 dilution in carbonate bicarbonate buffer, pH 9.6, was bound to 96-well flat bottom microtitration plates (ELISA Nunc Maxisorp, Nunc Inc., Rochester, N.Y., USA) by overnight incubation at room temperature. On cell culture plates, 50 µl of distemper virus and 50 µl of each serum dilution (0.9, 1.8 and 2.7) and respective controls were incubated for 1 hr at 37^0C with permanent shaking. After 3 washes, 50 µl of the virus-serum mix was transfered on the monoclonal antibody sensitized plates and incubated with shaking for 1 hr at 37^0C. Then, 50 µl of the monoclonal antibody marked with peroxydase were added in each well and plates incubated with shaking for 1hr at 37^0C. The plates were washed 3 times before 100 µl of substrate (orthphenylene diamine, Sigma Co.) was added. The reaction was stopped after 25 min with 50 µl of 2.5 M H_2SO_4 solution. Microtitration plates were read at 490 nm wavelength. Results were expressed as O.D. percent compared to the control without serum (100%). Serum titers were given as log of the reciprocal dilution with a 50% O.D. In order to validate the ELISA test and define the cut-off point (COP) for seropositivity, 23 serum samples of bears with an ELISA titer^{3}0.8 were also tested by the classical serum-neutralization (SN) test [4]. Titers by S.N. were usually lower than by ELISA, which led to defining the COP at ^{3}1.0. Furthermore, ELISA positive samples and a random sample of negative serum samples were also tested using an immunoperoxidase (IP) antibody test, which is similar to a fluorescence test, using IP instead of fluorescein isothyocyanate. (6) canine hepatitis virus by serum-neutralization, using CAV type 2 and dog kidney cell line MDCK (10^5 cells/ ml). Cytopathic effect on culture plates was read 7 days after infection, and titers were expressed in log10 protective dose 50% (PD50) on MDCK. Ages were estimated by examining cementum annuli of premolar teeth for BB and GB. BB were classified in 4 inclusive age groups : 0-2 yr old, 2.5-4 yr old, 4.5 -8 yr old and ^{3}9 yr old. GB were classified into 5 inclusive age groups : 0.5-2 yr (young bears), 2.5-4 yr (end of puberty,i.e subadult), 4.5-8 yr (reproductively mature bears), 8.5-12 yr (prime reproductive capability), and ^{3}13 yr (old bears). GB from the Seward Peninsula and McKinley Park were reported as being adults (i.e. ^{3}4.5 years). 62% (297/480) of the GB and 55% (22/40) of the BB were females. Among the GB, 70 (12%) were <2 year old, whereas 24 (31.5%) of the BB were <2 year old. Demographic data were analyzed using Epi Info 6.02. Frequency distributions were obtained and chi-square were calculated to obtain measures of association, and the statistical significance of such associations.

RESULTS

Overall seroprevalence in GB was 0% for CPV, 9% (43/480) for CDV, 14% (68/480) for CIHV, 16.5% (79/480) for brucellosis, 19% (93/480) for tularemia and 47% (225/478) for trichinosis. Seroprevalence in BB ranged from 0% for canine distemper and parvovirus to 32% (13/40) for tularemia (Table I). Canine parvovirus : None of the grizzly and black bears tested had canine parvovirus antibody at a significant titer.

Canine distemper : Antibodies were found only in GB, with an overall prevalence of 8.3% (40/480). Antibody prevalence was slightly higher in females (10%;29/294) than in males (6%; 11/186). Prevalence varied with the origin of the bears (Table I). GB from Kodiak Island and the Alaskan Peninsula were more likely than GB from other areas to be seropositive for canine distemper (RR=6.57, 95% CI=3.29, 13.12). Overall, among all 41 distemper positive samples, 73% had titers ^{3}2.0, and of these, 77% (23/30) were from southern Alaska. Animals with distemper positive samples were also more likely to be seropositive for CIH (RR=2.18, 95% CI=1.28, 3.72). Mean age of seropositive GB was 12.5 ± 0.85 yrs, whereas mean age of seronegative GB was 8.4 ± 0.27 yrs. Age prevalences for distemper antibody ranged from 1.5% in 0-2 yr-old GB to >10% in GB^{3}8.5 yr old. High titers were mainly observed in GB >8 yr old. In southwest and western Alaska, all seropositive GB were >7 yr old, whereas, in Northern Arctic, 40% of positive GB were £4 yr-old. Annualized prevalences for Kodiak Island were stable at 32% (1988) and 29% (1989). They ranged from 2.6% to 7.6% in Northern Arctic.

Canine infectious hepatitis : CIHV antibody prevalence was 14 % (68/480) in GB and 7.5% (3/40) in BB. Seroprevalence by sex was 12% (22/186) in males and 15.5% (46/294) in females for GB, and 5.5% (1/18) and 9% (2/22) for BB. Prevalence also varied with the geographical origin and the age of the bears. For GB, it was the highest on Kodiak Island (31%, 26/77), and averaged 10.5% in the other areas (range :7.5% to 12.5%). None of the young GB (0-2 yr old) had CIHV antibodies. Mean age of seropositive GB was 12.23 ± 0.57 yrs, whereas mean age of negative GB was 8.2± 0.28 yrs. Adult GB were more likely to be seropositive (RR=7.6, 95% CI=2.82,20.46) than young and subadult bears. Conversely, none of the adult BB were seropositive. Annual prevalences varied for BB from 0% in 1988 (0/9) and 1989 (0/22) to 4.5% (1/22) in 1990 and 8.7% (2/23) in 1991. For GB, overall prevalence decreased from 19% (38/202) in 1988 and 21% (24/114) in 1989 to 11% (13/121) in 1990 and 8% (11/134) in 1991. All BB tested more than once were seronegative. In GB, none of the 2 bears tested twice on Kodiak Island had CIHV antibodies. In Interior Alaska, of the 11 GB tested several times, one adult was persistently positive and 2 other adults were negative at the first collection and positive at the second. In Noatak river drainage area, of the 11 GB tested more than once, one female adult was consistently positive, another GB was negative at the first blood collection and strongly positive three years later. In the Arctic region, 5 adults were seropositive at both collections,

two adults were positive at the first blood collection and negative at the next ones, and one GB was initially negative and positive 3 years later.

Brucellosis : The overall prevalence of brucella antibodies was 16.5% (79/480) in GB and 2.5% (1/40) in BB. Antibody prevalence was similar in males (17%) and females (14.5%) for both species. Brucella prevalence varied widely by geographical areas (Table I). GB from western and northern Alaska (59/277) were more likely to be seropositive for brucellosis than GB from southwestern and central Alaska (20/203) (RR=2.16, 95% CI=1.35, 3.47). Mean age of seropositive GB (8.7± 0.59) was similar to the mean age of seronegative GB (8.8 ± 0.29). Seroprevalence increased from 6% in young GB (0-2yr old) to 17% in adults (≥4.5 yr old). Overall, the annual prevalence decreased from 19% (39/202) in 1988 to 6% in 1990 (7/121), but increased in 1991 to 14.5% (19/131). Yearly prevalence was consistent from one year to the other on Kodiak Island (12% (5/41) in 1988 and 8% (3/37) in 1989). A decrease was observed in Noatak river drainage from 37% (11/30) in 1988 to 15% (5/34) in 1991. In the Arctic region, yearly prevalence also decreased from 20% (14/69) in 1988 to 8% (5/65) in 1990, but re-increased in 1991 to 17% (13/76), mainly in the central and northwestern Arctic areas (38%, 8/21). Among the 78 GB tested more than once, 4 GB (5%) were consistently positive, 10 (13%) seroconverted and 7 (9%) became negative between the first and the following collections

Tularemia : The overall prevalence of *F. tularensis* antibody was 19% (93/480) in GB and 32% (13/40) in BB. Seropositive bears were also more likely to be positive for brucellosis (RR=2.33; 95% CI=1.57, 3.48). Seroprevalence was similar in males (22.5%) and females (19%). Prevalence varied from 4% (3/77) on Kodiak island to 10-15% in the Alaska Peninsula and Western Alaska and >30% in Interior Alaska and in the Arctic regions (Table I). Prevalence (32%) was identical in BB and GB from Interior Alaska. GB from Interior Alaska and from the Arctic regions (62/190) were more likely than GB from Western and Southwestern Alaska (31/290) to be seropositive for *F. tularensis* (RR=3.05, 95% CI=2.07, 4.51). In BB, antibodies were found only in BB <9 yrs old. Mean age of seropositive GB (7.33± 0.58) was slightly younger than mean age of seronegative GB (9.08± 0.29). Antibody prevalence in GB was the highest in the age group 2.5-4 yr old and the lowest in the 0-2 yr old group. Young and subadult GB(41/167) were 1.64 times more likely to be seropositive than adult GB (60/401) (95% CI=1.15, 2.34). On Kodiak Island, only 3 GB were seropositive with a low titer (1:20). GB with high titers (≥1:160) were mainly from the Arctic regions, Noatak river drainage and Interior Alaska. In Interior Alaska, the prevalence of bears with high titers was similar in BB (7/21) and in GB (4/15). In BB, annual prevalences decreased from 39% (12/31) in 1988-89 to 20% (9/45) in 1990-91. The same decrease was observed in GB from Interior Alaska (40% (6/15) in 1988-89 and 24% (9/38) in 1990-91). For all regions, the yearly prevalences in GB increased from 11% (36/322) in 1988-89 to 26% (65/252) in 1990-91. Among the 78 GB tested more than once, 42 (54%) were negative at all blood collections, 8 (10%) were positive at all blood collections, 10 (13%) were positive at the first blood collection and negative at the following ones, and 18 (23%) were negative at the first blood collection and positive at the following ones. Among the BB, 12 (48%) were negative and 5 (20%) were positive at all blood collections, 3 young BB were positive at the first collection and negative at the next one. One 1-yr old BB seroconverted the next year.

Trichinellosis : *Trichinella spiralis* antibody prevalence was 47% (225/478) in GB and 27.5% (11/40) in BB. Seroprevalence was higher (RR=1.21, 95% CI= 1.01, 1.46) in males (51%, 104/204) than in females (42%, 132/314). Prevalence varied geographically with an increasing gradient from Southwest Alaska to the Arctic. All the GB from Kodiak Island were seronegative and only one adult male from Katmai Coast (Alaska Peninsula) was positive, with a low titer (O.D.<0.6). Conversely, GB from Black Lake (Alaska Peninsula), Interior Alaska and Seward peninsula had a prevalence of 33% (42/128). Antibody prevalence was similar in GB (35%, 14/40) and BB (27.5%) from Interior Alaska. GB from Noatak river drainage had a prevalence of 57% (50/87) and GB from the Arctic regions had an overall prevalence of 89% (132/148). 70% of the GB with high titers (O.D.≥0.9) were from the Arctic regions and Noatak river drainage. In GB, antibody prevalence was similar in all age groups, with a mean age of seropositive GB (8.96 ±0.38 yrs) close to the mean age of seronegative GB (8.57±0.37). In BB, 10 of the 11 positive BB were <5 yr old. Yearly prevalences in GB were constant at Noatak river drainage (57% in 1988 and 1989, 59% in 1991) and decreased moderately in the Arctic regions (91% in 1988 to 80% in 1990-91). Of the 78 GB collected more than once, 50 (64%) tested positive and 18 (23%) tested negative at all blood collections; 10 (13%) were negative at one blood collection and positive at the other. For the 25 BB collected more than once, 17 (68%) tested negative at all collections, 5 (20%) BB became positive and 3 (12%) BB became negative at the second blood sample collection.

Table I

Prevalence of *Brucella*, *Francisella tularensis*, *Trichinella spiralis*, canine distemper virus and canine infectious hepatitis virus antibodies in black bears and Grizzly bears, Alaska, by geographical origin. 1988-1991

Game Area / (Species)	N	Brucella Pos. (%)	Tularemia Pos. (%)	Trichinella Pos. (%)	Distemper Pos. (%)	C.I. H. Pos. (%)
Kodiak Island (GB)	77	8 (10)	3 (4)	0 (0)	23 (30)	26 (31)
Katmai Coast/ Black Lake (GB)	86	11 (13)	12 (14)	17 (20)	7 (8)	8 (9)
Fairbanks/ Tanana Flats (GB)	40	1 (2.5)	13 (32)	14 (35)	1 (2.5)	5 (12.5)
Tanana Flats (BB)	40	1 (2.5)	13 (32)	11 (27.5)	0 (0)	3 (7.5)
Seward Pen. (GB)	40	10 (25)	4 (10)	12 (30)	1 (2.5)	3 (7.5)
Noatak Riv. Dr.(GB)	87	21 (24)	12 (14)	50 (57)	0 (0)	10 (11.5)
Arctic N.W. (GB)	96	15 (16)	34 (35)	86 (91*)	6 (6)	5 (5)
Arctic N.E.& C. (GB)	54	13 (24)	15 (28)	46 (83)	3 (5.5)	11 (20)

* Two young females not tested.

DISCUSSION AND REFERENCES : Available upon request from the Authors.